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Pharmacologic and Immunologic Approaches to the Problems of Posttraumatic Glial Proliferation Following CNS (Central Nervous System) Damage

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PHARMACOLOGIC AND IMMUNOLOGIC APPROACHES TO THE PROBLEMS OF POSTTRAUMATIC GLIAL PROLIFERATION FOLLOWING CNS DAMAGE

Melvin L. Billingsley[*], James P. O'Callaghan[#], and Carey D. Balaban[$]

Departments of Pharmacology (*) and Anatomy ($), The Milton S. Hershey Medical Center, Pennsylvania State University, Hershey, PA 17036, USA; and Neurotoxicology Division (#), Health Effects Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina, 27711, USA.

EPA Project Officer
James P. O'Callaghan

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ABSTRACT

We have devised a pharmacologic approach to block the proliferation of glial cells (gliosis) which follows various forms of trauma to nervous tissue. A method was devised using the incorporation of ^{3}H-thymidine incorporation into DNA of glial cells as a proliferative index following mechanical trauma. Both biochemical and histologic analysis revealed that treatment of lesioned rats with cell-cycle specific antimitotic agents such as cytosine arabinoside reduced glial proliferation as measured by both DNA synthesis and morphologic quantitation of cell numbers around the lesion. Treatment with cytosine arabinoside resulted in the appearance of numerous unusual cells near the lesion, with indications of aborted mitotic spindles in many of these cells. Other antimitotic drugs such as cyclophosphamide and vincristine were also able to inhibit glial proliferation. Additional experiments suggested that immunosuppression prior to lesioning greatly reduced the proliferative response of glial cells . We are also using treatments with neurotoxicants such as kainic acid and trimethyl tin to produce non-mechanical neuronal damage and subsequent gliosis. Preliminary results suggest that these toxicants reduce the immunocytochemical appearance of neuronotypic proteins, and that glial fibrillary acidic protein is markedly elevated in tissues which lose neurons. The relative merits of both mechanical and chemical models for inducing gliosis will be discussed, as will the therapeutic possibilities of selected antimitotic agents.

INTRODUCTION

Numerous severe pathologic changes occur following damage to the central nervous system (CNS) of mammals. Formation of a neuroglial scar following damage is seen throughout the neuraxis and is attributed to both hyperplastic (1) and hypertrophic responses (2) of non-neuronal cells near the lesion. One conceptual approach has been to consider the glial scar as a mechanical impediment for the regrowth of axons across this barrier (3,4). Thus, numerous attempts have been made to reduce the extent of the glial scar via surgical (5) and pharmacologic means (6). However, there have been few attempts to establish a rational pharmacologic approach to the problem of inhibition of glial scars. Clearly, attempts at pharmacotherapy must follow several guidelines which can established based on the metabolic differences between neurons and glial gells. The therapeutic modalities currently used in the treatment of head trauma focus primarily on reduction of brain edema (7) and maintence of adequate cardiovascular perfusion to the nervous system (8). Beyond this initial stage of therapeutic intervention, there are no currently acceptable pharmacologic approaches which can either prevent the induction of or inhibit neuroglial scar formation. The goal of this manuscript is to outline possible therapeutic modalities which may prove useful in the prevention of neuroglial proliferation following trauma. We will also explore several experimental models of in vivo glial cell proliferation which may prove useful in evaluating experimental therapeutic approaches.

Rationale for Pharmacologic Inhibition of Glial Proliferation

The prime consideration for a potential inhibitor of glial cell proliferation is that the agent specifically blocks formation of a glial cell scar without

compromising neuronal regrowth in the lesioned region. Two stages in the formation of a glial cell scar afford the best chance for therapeutic intervention. First, recent studies have suggested that lymphokines or similar mitogenic substances are secreted in response to brain injury and can induce the proliferation of astrocytes near the lesion (9-11). Thus, agents which specifically block this mitogenic activation may prevent the neuroglial scar from ever forming. Examples of therapeutic modalities active in this case would be immunosuppressive agents, or specific inhibitors of lymphokine receptor activation, such as antibodies against interleukins. A second stage for potential therapeutic intervention is the selective inhibition of glial DNA synthesis (12).

This latter approach assumes that following mitogen activation of astrocytes, new DNA sythesis occurs in glial cells near the site of the lesion (13). There are numerous antimitotic agents which can inhibit DNA synthesis; however, selectivity is a major problem. Intercalating and alkylating agents will effectively inhibit DNA synthesis, but will also affect the genomic DNA of neurons and other cell types (14). Since new DNA synthesis occurs during S phase of the cell cycle, agents which are selective for this particular phase would be more specific for mitogen-activated cells. Such agents include cytosine arabinoside (ara-C), Methotrexate (MTX), hydroxyurea, and 6-mercaptopurine (15). Of these agents, MTX and 6-mercaptopurine are considered to be self-limiting agents, which means that although these drugs act during S phase to inhibit DNA synthesis, they also interfere with RNA and protein synthesis, limiting the entry of additional cells into S phase (16). Since the majority of neurons in the adult CNS do not undergo mitotic division, S phase-specific antimitotic agents would not be expected to interfere with normal adult neurons (17).

Other cell-cycle-specific agents exist, such as the vinca alkaloids, vincristine and vinblastine which are specific for metaphase. Unfortunately, because of their effects on the cytoskeletal components of neurons, these agents produce unacceptable neurotoxicities, and are not logical choices for therapy of CNS damage (18).

One final concern involves the ability of the antimitotic agent to penetrate the blood-brain barrier following peripheral administration. Given the previous discussion, ara-C and hydroxyurea are the most likely candidates for parenteral therapy of glial proliferation. Hydroxyurea poorly penetrates the blood-brain barrier, necessitating administration into the ventricular system (19). Ara-C is able to penetrate the blood-brain barrier, and was used as a prototype agent for inhibiting posttraumatic glial proliferation (20).

A final category of agents which may exert selective effects on dividing glial cells would be inhibitors of polyamine biosynthesis, such as difluromethylornithine (DFMO). These agents irreversably inhibit ornithine decarboxylase, which is a key enzyme in the polyamine biosynthetic pathway, leading to an inhibition of cell growth (21).

METHODS

Models for Inducing in vivo Glial Proliferation

Mechanical trauma to regions of the central and peripheral nervous system of rats have been most often used to produce lesions which result in the formation of glial scars. Investigators have used transections of cranial nerves (22), spinal cord transections (23), and stab wounds of the cortex (24) to produce glial reactions following injury. Most often, ^{3}H-thymidine autoradiography has been used to document a mitogenic response of glial cells (25). Other investigators have examined the accumulation of glial fibrillary acidic protein (GFAP) around lesions using immunocytochemical techniques (26). For some of the pharmacologic studies of the effects of DNA synthesis inhibitors on posttraumatic glial cell proliferation, we developed a biochemical method which could be used to determine the extent of new DNA synthesis in the lesioned CNS (27). By examining the incorporation of intraventricularly administered ^{3}-thymidine into DNA extracted from mechanically lesioned and control brain tissue, we we able to develop an index of DNA synthesis following damage.

Administration of specific neurotoxicants can also result in reactive proliferation of glial cells. Direct injections of kainic acid in regions of the brain results in the local destruction of neuronal cell bodies and a resultant increase in glial proliferation (28). We have also administered the neurotoxicant trimethyl tin to rats, which produces a characteristic pattern of neuronal loss and susequent gliosis in the hippocampus and limbic regions of the brain (29). By examining several experimental models which produce glial proliferation, we hope to develop an understanding of the mechanisms and possible treatments which can prevent further loss of function as a result of CNS damage.

Induction of Glial Proliferation: Mechanical lesions were produced in anesthetized adult rats by using an 18 gauge needle to damage the top 2 mm of frontal cortex as described (30). Surface coordinates were used to determine the site of the lesion, using 1 mm lateral and anterior of bregma as the site for lesioning.

Determination of Glial DNA Synthesis: On day 2 post lesion, which was determined to be the peak time of DNA synthesis in the lesioned cortex, ^{3}H-thymidine (48 Ci/mmol; Amersham) was administered directly into the lateral ventricles (20 uCi in 20 ul saline), and the animals were sacrificed 60 min later. Brain DNA was extracted into ice-cold 0.2 N perchloric acid, centrifuged, and an aliquot of the acid-soluble pool was used to determine total unincorporated tritium in the tissue. The pellet was delipidated, and the DNA hydrolysed in 0.5 N perchloric acid heated to 90^{0} C. An aliquot was used to determine ^{3}H-thymidine incorporation, and another aliquot used to determine DNA content. Data were expressed as a fractional index of radioactivity incorporated into DNA per amount of total tissue radioactivity (31). This method accounts for unequal distribution of radioactivity to the tissue, and with respect to drug studies, allows for measurement of DNA synthesis which is independent of drug-induced changes in DNA content.

Drug Treatments: Rats were mechanically lesioned as described above, and treated for two days post lesion with either ara-C (100 mg/kg/day, i.p.);

methotrexate (200 mg/kg/day, i.p.); cyclophosphamide (75 mg/kg/day, i.p.); vincristine (1.0 mg/kg/day, i.p.) or vinblastine (1.0 mg/kg/day, i.p.). On day two post lesion, rats were intraventricularly injected with ^{3}H-thymidine, and DNA synthesis was determined in the lesioned and control frontal cortices.

Kainic Acid Lesions: Rats were anesthetized, mounted in a stereotaxis and 2.0 ug of kainic acid was directly injected into the left corpus striatum. Two days following the lesion, surviving rats were injected with apomorphine (1.0 mg/kg, s.c.) and successful striatal lesions were confirmed by recording the number and direction of rotation following stimulation of the surviving right striatum (32).

Histological Analysis: Several groups of Rats received mechanical lesions as described above, were perfused with 10% buffered formalin and processed for parrafin-embedded histological sections. Lesioned and control cortical tissues from saline and ara-C-treated rats were sectioned into 8 um coronal slices, mounted on gelatin-coated slides, and stained with either Luxol fast blue with neutral red counterstain, thionin, hematoxylin and eosin, or the Sevier Munger silver stain. Cellular morphology was examined and neurons and glial cells were counted in ten fields adjacent to the lesion, using an 0.1 x 0.1 mm ocular grid under oil immersion (1000x). All slides were coded and examined in a single blind design. Significant differences were determined using Student's paired t-test.

Administration of Trimethyl Tin: Adult rats were injected with trimethyl tin (3,6 and 9 mg/kg, i.v.), and were sacrified three weeks following this single dose. Neonatal rats were treated with trimethyl tin on postnatal day 5 (6.0 mg/kg, i.p.), and sacrificed on postnatal day 66. Animals which were used to determine levels of GFAP were sacrificed by decapitation; animals used for immunohistochemical procedures were perfused with cold phosphate-buffered saline, followed by phosphate-buffered 10% formalin (33).

Measurement of GFAP: In animals receiving trimethyl tin, GFAP was measured using a solid-phase immunoassay (dot-blots) as described (34). Hippocampal sections were homogenized in 50 mM Tris, 150 mM NaCl buffer (pH 7.4) , and applied to nitrocellulose filters. Excess protein binding sites were blocked with 0.5% gelatin and anti-GFAP (Dako), was incubated with the filter (1:500). The GFAP antibody was quantified with ^{125}I-protein A, using purified GFAP as a standard.

Immunohistochemistry of Rats Treated with Trimethyl Tin: In order to document changes in neuronal populations following lesions with trimethyl tin, immunocytochemical analysis of the distribution of the enzyme protein-O-carboxylmethyltransferase (PCM; E.C.2.1.1.24) was examined: this enzyme is located primarily in neurons throughout the nervous system and can be considered a neuronotypic protein (35,36). A vibratome was used to produce 50-100 uM sections from formalin-fixed brains. Coronal sections were taken through the limbic and hippocampal regions. Following equilibration in phosphate-buffered saline (50 mM phosphate buffer, 0.9% NaCl, 0.5% triton X-100), the sections were incubated overnight with antiserum against PCM, followed by detection using a biotinylated goat antirabbit IgG and avidin-horseradish peroxidase; diaminobenzidine was used as a chromogen. The specificity of this antiserum has been previously described (35). Stained tissues sections were photographed using Kodak Technical Pan Film at ASA 25 using a green filter.

RESULTS

Inhibition of ^{3}H-thymidine Incorporation into Glial DNA by Antimitotics: We have found that rats receiving mechanical lesions of the frontal cortex incorporated significantly more ^{3}H-thymidine into the DNA of the lesioned cortex than that of the control frontal cortex. DNA synthesis was significantly elevated during the first four days following the lesion, with peak levels occurring on day 2 post lesion (30). The rate of incorporation remained linear during the first 75 min following intraventricular injection of ^{3}H-thymidine. Thus, the effects of antiproliferative drugs were examined during the first two days following the lesion, 60 min after injection of ^{3}H-thymidine. Table 1 shows the effects of antimitotic drug treatment during the first two days following mechanical cortical lesions on the rate of DNA synthesis in glial cells . Treatment with cytosine arabinoside (100 mg/kg/day), cyclophosphamide (75 mg/kg/day), or vincristine (1 mg/kg/day) significantly inhibited DNA synthesis in the lesioned frontal cortex. Treatment with methotrexate (200 mg/kg/day) or vinblastine (1 mg/kg/day) did not inhibit DNA synthesis in the lesioned cortex. The degree of inhibition of DNA sythesis paralleled the known potency of the compounds, and the lack of inhibition seen following treatment with methotrexate and vinblastine correlates with their inability to enter the CNS following peripheral administration.

Histologic Evidence for Inhibition of Glial Cell Proliferation by Ara-C: The inhibition of glial cell proliferation by ara-C was also corroborated using counts of glial cells in 10 random microscope fields (0.1 x 0.1 mm ocular grid) within 1 mm of the lesion; similar counts were performed on the contralateral control cortex. These results were obtained for a total of 72 coded slides, which were read in a single-blind manner. Rats received mechanical lesions as described above, and were treated with either saline or ara-C (100 mg/kg/day; i.p.)for 4 days following the lesion. Groups of animals were perfused for histology on days 4 and 12 post lesion, and paraffin-embedded sections were stained with thionin or Luxol fast blue-neutral red as described. Figure 1 shows the effects of saline and ara-C treatments on animals examined four days post lesion. Animals treated with saline showed a significant increase in glial cells near the lesion relative to the contralateral control cortex. Animals treated with ara-C did not have this same increase in glial cell numbers. By day 12 post lesion, saline-treated rats had an even greater number of glial cells near the lesion (Figure 2). Animals treated with ara-C for four days post lesion had significantly less numbers of glial cells than that of saline-treated animals; however, there was a trend which suggested that a rebound proliferation was occurring during days 4-12 post lesion when ara-C was not administered. These data support the biochemical evidence suggesting that treatment with ara-C following neural lesions reduces glial DNA synthesis and subsequent proliferation of glial cells.

Figures 3 and 4 show the effects of ara-C on the histology of both lesioned and control cortical tissues. In Figure 4, a low-power (200 x) micrograph of saline treated (lesion-saline), and ara-C-treated tissues are shown (Lesion-ara-C and control-ara-C). The micrographs show the regions just below the margin of the lesioned frontal cortex, and the same control region of the contralateral control cortex; the small black stars show numerous cells in aborted mitoses which were only found in the lesioned cortex of ara-C treated rats. Figure 4 affords a closer view of these unusual cells in the ara-C treated lesioned frontal cortex. In panel A, (400 X) numerous large cells with abnormal mitotic

figures can be seen throughout the tissue. A higher magnification of these cells are shown in Panels B-D; abnormal chromatin is prevalent in all of the cells. The vaculated appearance of these cells is suggestive of an abortive mitosis caused by DNA synthesis inhibition.

Ornithine Decarboxylase Inhibitors and Glial Proliferation: As an alternative to antimitotic agents which directly affect DNA synthesis, we attempted to use the irreversable inhibitor of ornithine decarboxylase, difluromethylornithine (DFMO) to inhibit enhanced DNA synthesis following mechanical lesions of the frontal cortex. Animals were treated with DFMO (500 mg/kg/day; i.p.) for two days post lesion, and DNA synthesis was determined as described. Figure 5 shows the lack of effect of DFMO on the enhanced DNA synthesis in the lesioned cortex, suggesting that inhibition of ornithine decarboxylase is not sufficient to inhibit glial DNA synthesis following neural trauma.

Effects of Kainic Acid on Glial Proliferation: Kainic acid is a potent neurotoxin which destroys neurons in localized brain regions but spares axons of passage following direct intracerebral injection. Figure 6 shows the effects of intrastriatal injection of 2.0 ug kainic acid on striatal DNA synthesis, striatal wet weight, body weight, and the extent of apomorphine-induced turning in animals surviving for two days. There was a significant increase in DNA synthesis and wet weight in the lesioned striata, indicative of both reactive gliosis and severe edema. There was a decline in body weight following the lesion, and all surviving rats exhibited apomorphine-induced rotation, indicative of a successful striatal lesion. However, analysis of the acid-soluble pool of radioactivity in kainate-treated rats showed profound changes relative to saline-treated animals (Table 2), and suggests that the distribution of of intraventricularly administered ^{3}H-thymidine following kainic acid administration is grossly altered. Thus, we have chosen not to use kainic acid as a means of producing experimental gliosis.

Neuronal Damage and Glial Proliferation Following Trimethyl Tin Administration in Adult Rats: Trimethyl tin is an organometallic compound which produces a characteristic pattern of damage in the hippocampus and limbic regions of the brain. We have utilized this toxicant as a chemical model for neurotoxicity and the induction of glial proliferation. Figure 7 shows the morphology of the hippocampus of adult, Long-Evans rats dosed with trimethyl tin (6 mg/kg; i.v.) 21 days prior to sacrifice (cresyl violet stain). Note the damage to neurons in the CA fields, and the increased numbers of cell bodies in this region. The hippocampal content of GFAP was measured using a solid-phase immunoassay in toxicant-treated rats. The amount of immunoreactive GFAP in the hippocampus increased in a dose-dependent manner following trimethyl tin administration (Table 3). The dose of 6 mg/kg was the lowest dose which produced a significant increase in GFAP and a significant decrease in hippocampal wet weight. When the hippocampi of trimethyl tin-treated rats were microdissected into CA1, CA2 and CA3 fields, large increases in GFAP immunoreactivity were observed, and this is shown in Table 4. These data suggest a reactive gliosis following treatment with this neurotoxicant.

We used immunocytochemistry of protein-O-carboxylmethytransferase (PCM) to document the neuronal damage caused by trimethyl tin administration in adult rats. PCM immunoreactivity in control sections of hippocampus and temporal cortex is shown in Figure 8. PCM is located in neurons throughout the hippocampus and cortex, and delineates all of the major neuronal cell types in the hippocampal region. The administration of trimethyl tin (6 mg/kg; i.v.) 21

days prior to sacrifice produced substantial changes in the pattern of immunoreactive PCM in the hippocampus (Figure 9) and the temporal cortex (Figure 10) of adult rats. In the hippocampus, immunoreactive PCM was punctate and did not clearly define neural soma (Figure 9 A-D); this alteration was seen throughout the hippocampus, indicating that this neurotoxicant produced a loss of neurons. The immunoreactive PCM in the temporal cortex of adult rats treated with trimethyl tin was clearly abnormal when compared with that seen in control tissues (see Figure 8B). There was a generalized loss of PCM in all layers of the temporal cortex, indicating neuronal loss in this region. Of the cells that were positive for PCM (Figure 10B), most were abnormal in morphology, which again indicates that trimethyltin was responsible for neuronal destruction. When PCM immunoreactivity was examined in the substantia nigra of trimethyl tin-treated adult rats, no real differences were seen between treated and control tissues (Figure 11). This indicates that the alterations in PCM immunoreactivity in the hippocampal and temporal lobe regions were the result of the neurotoxic lesions caused by trimethyl tin, and not due to an interaction of trimethyl tin with PCM.

When neonatal rats were treated on postnatal day 5 with trimethyl tin, a marker reduction in cortical size was obvious. In some animals the hippocampal formation was absent, and the histology of remaining limbic system neurons indicated severe abnormalities.

DISCUSSION

This manuscript presents several aspects of research which are concerned with the experimental production and treatment of posttraumatic glial cell proliferation in rats. The goal of such research is to produce a comprehensive approach to the treatment of glial scar formation after damage to the CNS. Several points emerge from these studies concerning potential models and treatments.

First, several very different forms of neural insult appear to result in the formation of glial scar. We have found that mechanical lesions of the cortex result in a gliosis near the site of trauma, with DNA synthesis and increased numbers of glial cells seen within four days of lesioning. Similarly, direct administration of kainic acid into the striatum of the rat resulted in significant neuronal damage, an increase in DNA synthesis and considerable edema. Systemic administration of a specific neurotoxicant, trimethyl tin, was also found to produce neuronal damage and subsequent gliosis. Each model has advantages and disadvantages for the reliable production of CNS damage and subsequent gliosis. Mechanical lesions of the cortex can be easily produced; however, they result in disruption of the blood-brain barrier, which can result in the infiltration of monocytes. Mechanical trauma most closely resemble damage caused by penetrating wounds in humans. Kainic acid will directly destroy neuronal cell bodies while sparing axons of passage. However, the injection produces a rather high rate of mortality (20-50%) and also disrupts the blood-brain barrier. The edematious changes in both the lesioned and contralateral control striatum is unusual, in that much more soluble radioactivity is found in the basal ganglia following intraventricular administration of radioactivity. Thus, this model was not used for studies involving DNA synthesis and subsequent evaluation of drug effects. There is no human counterpart to this model of CNS damage.

Administration of trimethyl tin, a selective neurotoxicant, also produced a characteristic pattern of neuronal damage and gliosis; however, the time frame of toxicity is such that this model is less suited for the study of DNA synthesis. Immunocytochemical evaluation of gliotypic (GFAP) and neuronotypic (PCM proteins can be easily used in this model. There is no disruption of the blood-brain barrier, and the pattern of damage can be reliably predicted. The large increases in GFAP coupled with losses in the immunochemical pattern of PCM staining in the hippocampus provides a specific histologic index by which damage can be assessed. It is of note that the neurotoxicant model of damage and gliosis has a human counterpart , namely, accidental exposure to neurotoxic agents.

Thus, the model to be used for examination of the potential therapeutic effects of drugs on the glial reaction to injury needs to take in to account some of the advantages and liabilities of each method. We feel that the mechanical model of trauma is appropriate for pharmacologic studies which use biochemical endpoints while the neurotoxicant-based models are better suited for immunochemical assays.

Several antimitotic agents effectively inhibited lesion-induced increases in DNA synthesis. Of these, only ara-C met the criterion of glial versus neuronal selectivity. Cyclophosphamide crosses the blood-brain barrier, and was effective as an inhibitor of DNA synthesis in both the lesioned and control corticies. However, this agent can affect cells in any stage of the cell cycle. Vincristine was also effective as an inhibitor of DNA synthesis in the lesioned cortex; this agent produces unacceptable neurotoxicities. Neither methotrexate nor vinblastine affected DNA synthesis, an effect most likely related to their

inability to access the brain. Thus, ara-C appears to be the most appropriate category of drug for the prevention of glial proliferation. It is S-phase specific, and should not harm neurons. Ara-C can penetrate the blood-brain barrier, but can also be centrally administered in order to prevent systemic toxicities . The effects of ara-C were also investigated using quantitative histology. This drug was found to inhibit the increase in glial cell numbers at 4 and 12 days post lesion, suggesting that DNA systhesis inhibition led to a decreased number of glial cells near the lesion. Also, numerous unusual cells were observed near the margin of the lesion in rats treated with ara-C. These cells appeared to have undergone an abortive mitosis and were misshapen in appearance. It is possible that these cells were reactive glial cells which were inhibited by ara-C. Other investigators have subsequently used ara-C to inhibit glial proliferation following optic nerve transection, and found that ara-C was able to inhibit the proliferation of glial cells in this system, suggesting that this drug can be used in a number of different types of CNS trauma (37).

An additional pharmacologic approach was to use DFMO, an ornithine decarboxylase inhibitor, to attempt to reduce glial DNA synthesis following mechanical lesions. This agent did not significantly affect DNA synthesis in the lesioned cortex. In addition, measurement of the activity of ornithine decarboxylase in the lesioned and control cortex revealed no significant differences between lesion and control. Thus, this class of agent is not expected to prove useful in the treatment of posttraumatic gliosis. Also, neurons utilize polyamines in various reactions which are not related to DNA synthesis, and this agent would be expected to inhibit the functioning of polyamine pathways in neurons as well as in glia.

We are currently using neuronotypic and gliotypic proteins as markers for CNS damage and the reactive gliosis which accompanies the damage (38). We have found that PCM, a protein methyltransferase, can serve as a useful index of neuronal damage. Similarly, GFAP immunoreactivity can be used as an index of glial reactivity. We are in the process of using this combined immunohistochemical approach to examine the effects of drugs such as ara-C on the reactive gliosis which accompanies CNS damage. It is hoped that the use of immunocytochemical and biochemical indices will provide insights into the mechanisms and possible treatments for inhibition of posttraumatic glial cell scar formation.

In a separate study, we described experiments which indicated that the immune system was involved in the initiation of the increased DNA synthesis which follows CNS damage (10). Animals which were severely immunosuppressed using pretreatments with either hydrocortisone or methotrexate did not have any increased DNA synthesis in the lesion. We interpreted this finding as evidence for a role of the immune system in the induction of glial prolifeeration. Subsequently, other investigators have demonstrated that interleukin-2 (11) and other monocyte-derived factors (9) can trigger glial mitogenesis in vitro. Thus, the suggestion has been made that microglial cells secrete factors which initiate astrocyte reactivity. The interface between the immune system and glial cells will be the source of interesting studies in the future. It is hoped that this discussion will provoke interest into the potential therapeutic applications which can inhibit gliosis and hopefully, allow some restoration of function to the damaged brain.

FIGURE LEGENDS

Figure 1:Effects of ara-C on the number of glial cells 4 days post lesion. Rats received a mecahnical lesion of the frontal cortex, and were treated with ara-C (200 mg/kg/day; i.p.) or saline during the first 4 days post lesion. On day 4, the brains were prepared for histology using thionin or Luxol fast blue/neutral red staining procedures. Glial cells were determined in 10 random ocular fields (0.1 x 0.1 mm ocular grid) under oil immersion (1000X) within 1 mm of the lesion or the same region of the contralateral control cortex. Slides were examined in a single blind manner, and when the code was broken, results analysed using Student's paired t-test, lesioned versus control cortex.

Figure 2: Effects of ara-C on the number of glial cells 12 days post lesion. Rats received a mechanical lesion of the frontal cortex, and were treated with ara-C (200 mg/kg/day; i.p.) or saline during the frist 4 days post lesion. Drug treatment ceased on day 4 post lesion and on day 12 post lesion, the brains were removed and prepared for histology using either thionin or Luxol fast blue/neutral red staining. Glial cells were determined in 10 random ocular fields (0.1 x 0.1 mm) under oil immersion (1000X) within 1 mm of the lesion and the same region of the contralateral control cortex. Slides were examined in a single blind manner, and when the code was broken, results were analysed using Student's paired t-test, lesioned versus control cortex.

Figure 3: Histology of rat brain frontal cortex following mechanical lesions. A representative slide used for quantitative histology is shown, comparing lesion sites in control and ara-C treament groups. The contralateral control cortex of ara-C treated rats is shown in the last panel (control-ara-C). Luxol fast blue with neutral red counterstain (200X).

Figure 4: Formation of unusual mitotic figures following ara-C treatment of rats with lesioned frontal corticies. Panel A- cortical field approximately 1 mm below lesioned cortex, following treatment with ara-C (200 mg/kg/day) during the first 4 days post lesion (400X). Panels B-D- Oil immersion view of abnormal cells near the lesion of ara-C treated rats; arrows show cells which appear to have undergone abortive mitosis (1000X).

Figure 5: Effects of difluromethylornithine treatment (DFMO) on glial DNA synthesis in rats receiving lesions of the frontal cortex. Rats were mechanically lesioned, and treated with DFMO (500 mg/kg/day; i.p.) during the first two days post lesion. DNA synthesis was measured on day 2 post lesion, following intraventricular injection of ^{3}H-thymidine (20 uCi) in saline treated and DFMO treated rats. Each bar represents the mean and the standard error of the mean (n=10), using Student's paired t-test to determine significance (lesion vs control. The second panel shows the activity of ornithine decarboxylase in the lesioned and control cortex of saline-treated rats on day 2 post lesion. Activity was measured as pmol CO_2 evolved/mg protein/min.

Figure 6: Effects of intrastriatal injection of kainic acid on DNA synthesis (FI), body weight, striatal wet weight and apomorphine-induced turning. Rats received 2.0 ug kainic acid directly into the striatum, and on day 2 post lesion, received apomorphine to verify the success of the lesion. On day 3, rats were intraventricularly injected with ^{3}H-thymidine (20 uCi) and DNA synthesis was determined 60 min later (FI). Significant differences were determined using

Student´s paired t-test; lesion versus control striatum.

Figure 7: Effects of trimethyl tin administration on hippocampal morophology in adult rats. Rats received trimethyl tin (6.0 mg/kg; i.v.) and 21 days post lesion, brains were fixed, removed, cut into 10 um sections, and stained with cresyl violet. In control rats, a darkly stained group of cell bodies can be seen throughout the CA fields and in the dentate gyrus. treimethyl tin treatment (TMT) resulted in a loss of staining in all CA fields. (magnification =100 X).

Figure 8: Protein-O-carboxylmethyltransferase immunoreactivity (PCM) in hippocampus (A) and temporal cortex (B). Formalin-fixed brains of control rats were cut into 50-100 um coronal sections, and were immunostained for PCM as described in Methods. Note the intense staining of neuronal cells in all regions of the hippocampus (A). Neurons are also stained in temporal lobe cortical regions (B). 100 X.

Figure 9: Effects of trimethyl tin administration on PCM immunoreactivity in adult rat hippocampus. Adult rats were treated with trimethyl tin (6 mg/kg;i.v.) and were sacrificed and perfused on day 21 post dosing. Formalin-fixed brains were cut into 50-100 um sections and PCM immunoreactivity was determined as described in Methods. Panel A- diffuse PCM immunoreactivity in the molecular layer of the hippocampus; Panel B- reduction in neural PCM immunostaining in CA fields of the hippocampus. Panels C, D- Loss of cellular patterns of PCM immunoreactivity in the hippocampus; compare with the patterns seen in control hippocampus (Figure 8) .

Figure 10: PCM immunoreactivity in the temporal lobe of adult rats treated with trimethyl tin. Panel A- PCM immunoreactivity is greatly reduced following trimethyl tin administration (6 mg/kg; i.v.) 21 days prior to immunocytochemistry. Panel B- Higher magnification of PCM-positive neurons in the temporal cortex.

Figure 11: PCM immunoreactivity in the substantia nigra of control and trimethyl tin-treated adult rats. Adult rats were treated with trimethyl tin (6 mg/kg; i.v.) and were prepared for immunocytochemistry 21 days later. There were no aparrent differences in PCM immunostaining in this region.

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TABLE 1

EFFECTS OF SEVERAL ANTIMITOTIC AGENTS ON POSTTRAUMATIC DNA SYNTHESIS IN BRAIN

TREATMENT	DOSE (mg/kg)	INHIBITION OF DNA SYNTHESIS (%) Lesioned Cortex	Control Cortex
ara-C	100	90%	50%
cyclophosphamide	75	90%	55%
vincristine	1	80%	10%
vinblastine	1	0%	0%
methotrexate	200	0%	0%
saline	-	0%	0%

Adult rats recevied a stab lesion on the left frontal cortex two days prior to determination of DNA synthesis. Animals were treated with various antimitotic agents at the stated doses each day after the lesion. One hour following intraventricular administration of ^{3}H-thymidine, brain DNA was extracted and DNA synthesis determined using a fractional index for incorporation. Results are expressed as a percent inhibition of DNA synthesis versus saline-treated tissues. Each group contained at least 10 rats. Ara-C, cyclophosphamide and vincristine significantly inhibited DNA synthesis in the lesioned cortex ($P < 0.05$; Student's paired t-test).

TABLE 2

INCORPORATION OF ^{3}H-TdR INTO STRIATAL DNA AND ACID-SOLUBLE RADIOACTIVITY IN NORMAL AND KAINIC ACID-LESIONED RATS

Treatment	Fractional Incorporation		Acid-Soluble ^{3}H (DPM/µg DNA)	
	Left Striatum	Right Striatum	Left Striatum	Right Striatum
Saline	6.69 ± 0.82[b]	9.00 ± 1.39[b]	118.9 ± 14.1	199.5 ± 54.1
Kainic acid (2 ug)	27.38 ± 3.66[c]	21.91 ± 2.30	5831.0 ± 2406.0[a]	7297.0 ± 2040[a]

Rats were injected with kainic acid directly into the striatum. On day 2 post lesion, rats received apomorphine (1.0 mg/kg, s.c.) to verify the success of the kainic acid lesion. On day 3 post lesion, the rats were injected with ^{3}H-TdR (20 µCi; i.v.t.), and the FI was determined 60 min later. The left striatum of kainic acid-treated rats was the lesioned side. The saline group represents the baseline incorporation of ^{3}H-TdR in unlesioned rat striata, and was used for comparison with lesioned rats.

a = $P<0.001$ compared with saline treatment levels of ^{3}H.

b = $P<0.01$ compared with left and right kainic acid-lesioned striata, respectively.

c = $P<0.05$ comparing the left (lesioned) striata with the right (unlesioned) striata following kainic acid treatment, day 3.

TABLE 3

GFAP Immunoreactiviy in hippocampus of adult rats treated with TMT

Dose of TMT (mg/kg)	Hippocampus Wts. (g)	GFAP (% of control) per hippocampus	GFAP (% of control) per mg protein
0.0	.055±.004	100± 7	100± 6
3.0	.053±.001	99± 9	100± 9
6.0	.045±.002*	251± 25*	331± 43*
8.0	.044±.003*	303± 27*	372± 38*
9.0	.038±.002*	412± 42*	586± 60*

Rats were killed 3 weeks after treatment; each value represents the mean + SEM for 6 independent measurements.

*Significantly different from control $P < .01$

TABLE 4

Effect of TMT on GFAP in regions of dorsal hippocampus

	GFAP immunoreactivity (percent of control)		
	CA 1	CA 2	Fascia Dentata-CA 3
Control	100+ 38	100 ± 35	100 ± 14
TMT	*1571± 260	*5788 ± 640	*1248 ± 430

Each value represents the mean + SEM for 6 independent measurements obtained three weeks following exposure of rats to TMT (8.0 mg/kg) or saline (0.9%) vehicle (control)

*Significantly different from control, $P \leq 0.01$

For measurements of GFAP, 700 uM thick sections of dorsal hippocampus were prepared with a McIlwain Tissue Chopper and subdivided into the major hippocampal fields, CA1, CA2 and CA3/Fascia Dentata. Individual regions from 5 slices were pooled for subsequent assay.

MEAN NUMBER OF GLIA/CORTICAL FIELD—FOUR DAYS POST LESION

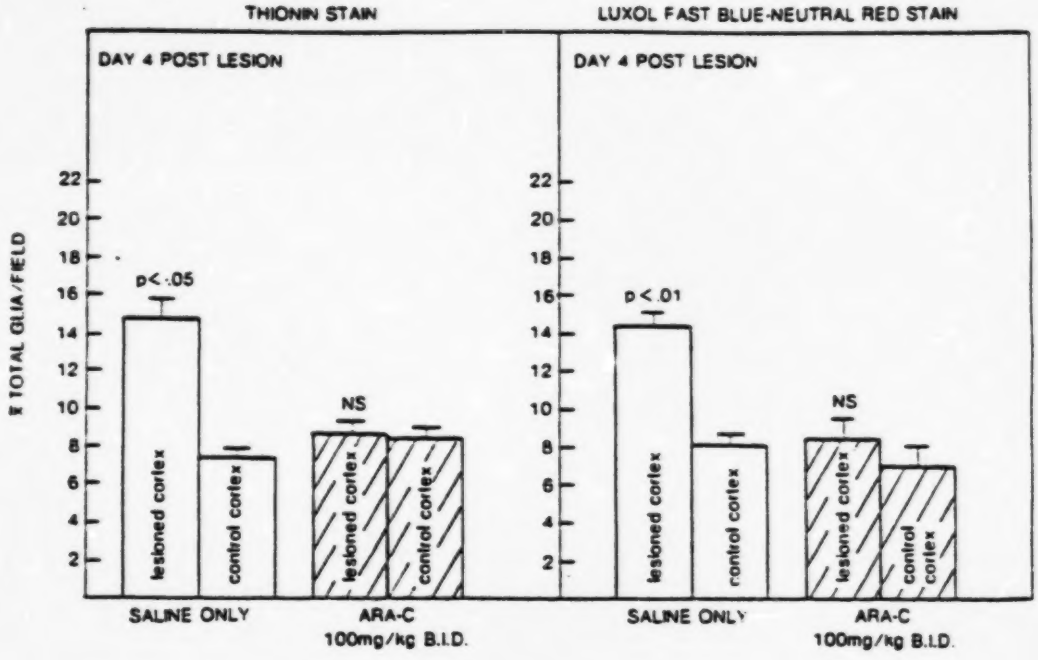

Fig 1

MEAN NUMBER OF GLIA/CORTICAL FIELD—TWELVE DAYS POST LESION

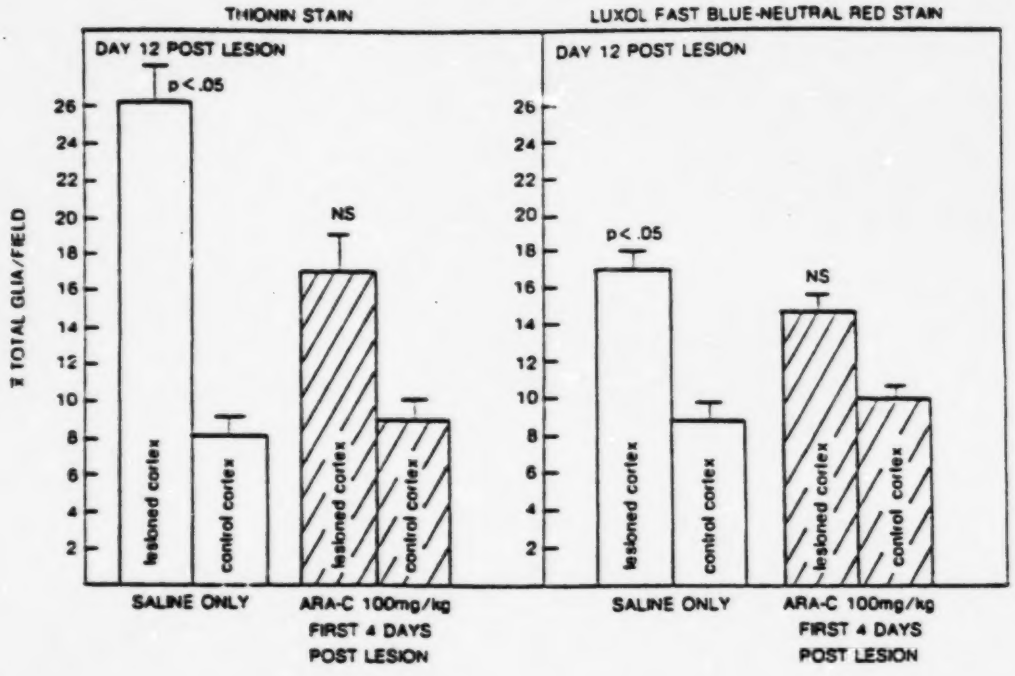

FIGURE 2

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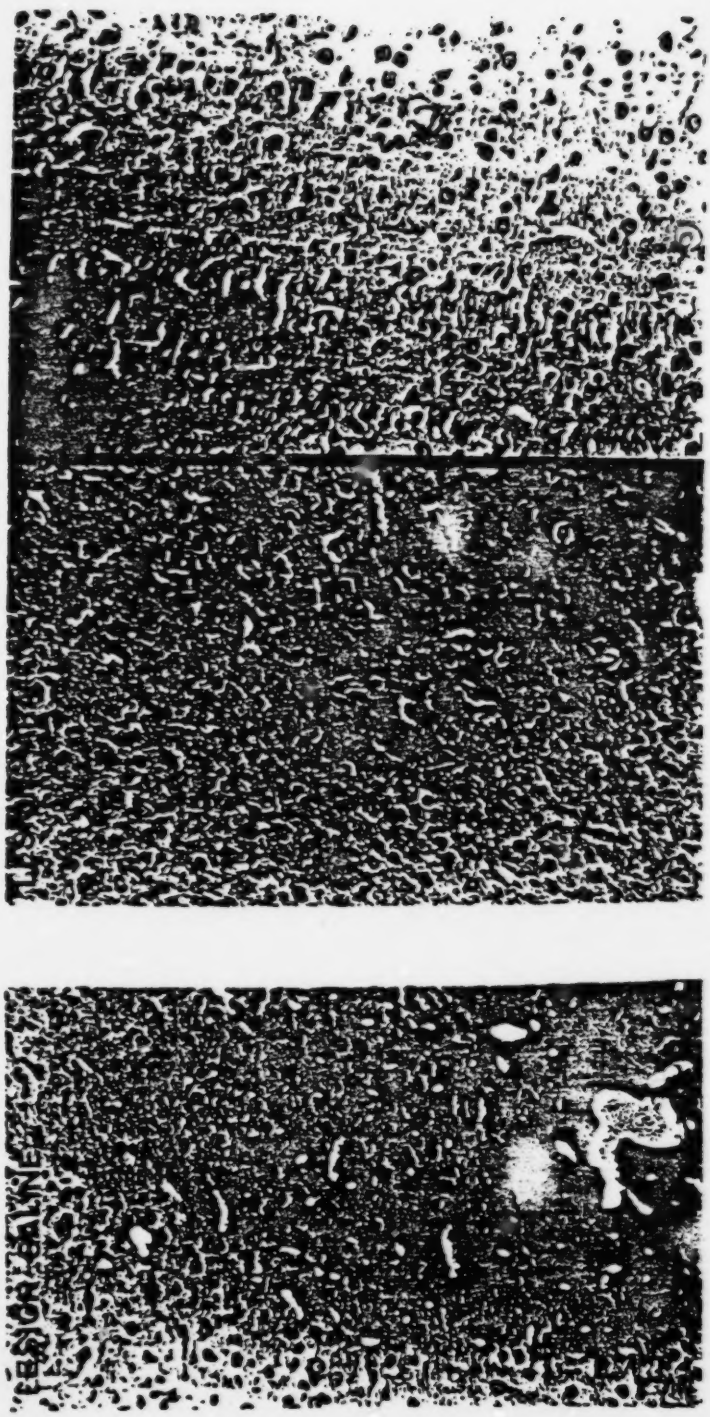

FIGURE 3

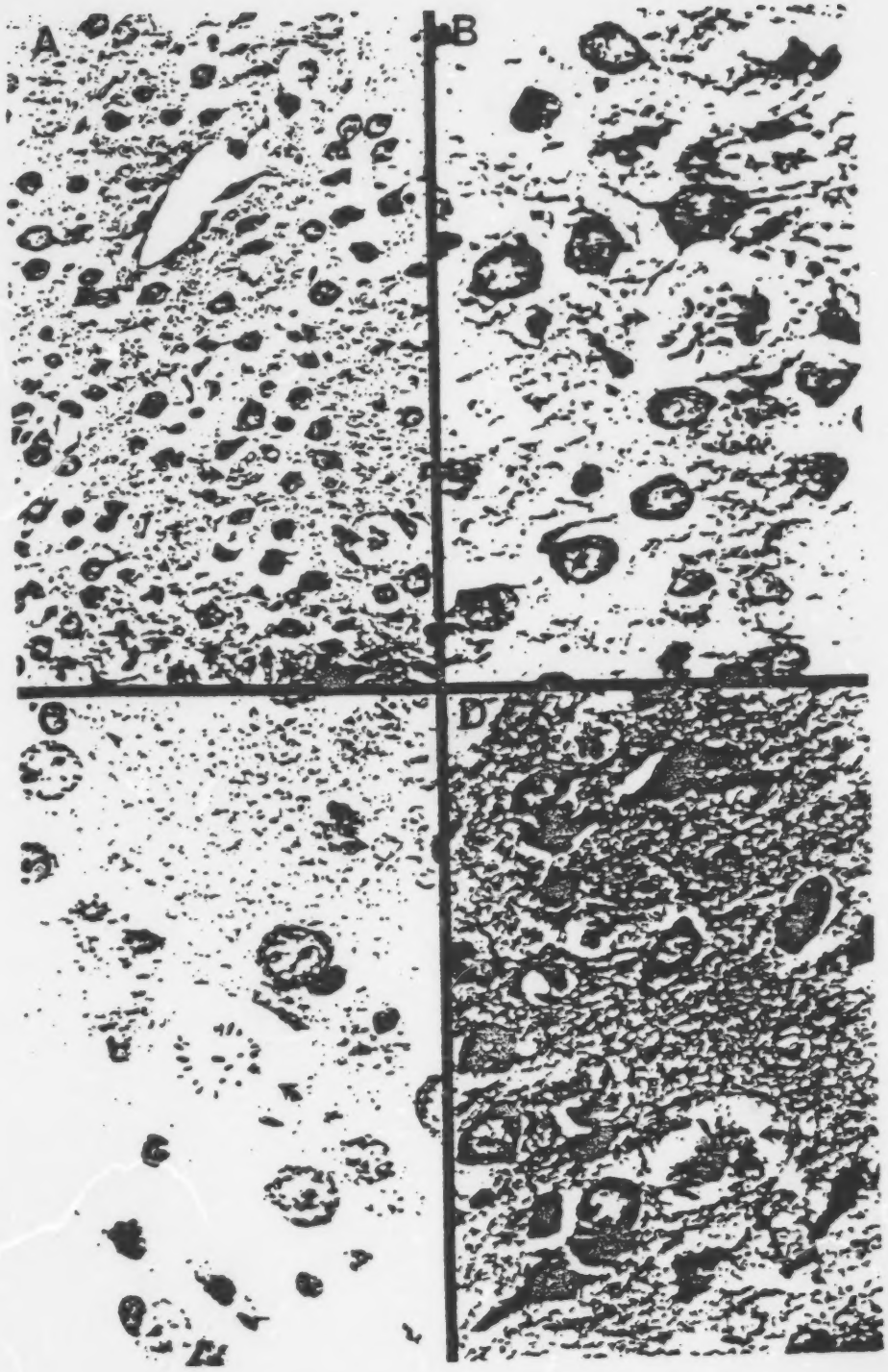

FIGURE 4

EFFECTS OF INTRASTRIATAL KAINIC ACID ON FRACTIONAL INCORPORATION AND STRIATAL WET WEIGHT BODYWEIGHT AND APOMORPHINE-INDUCED ROTATION

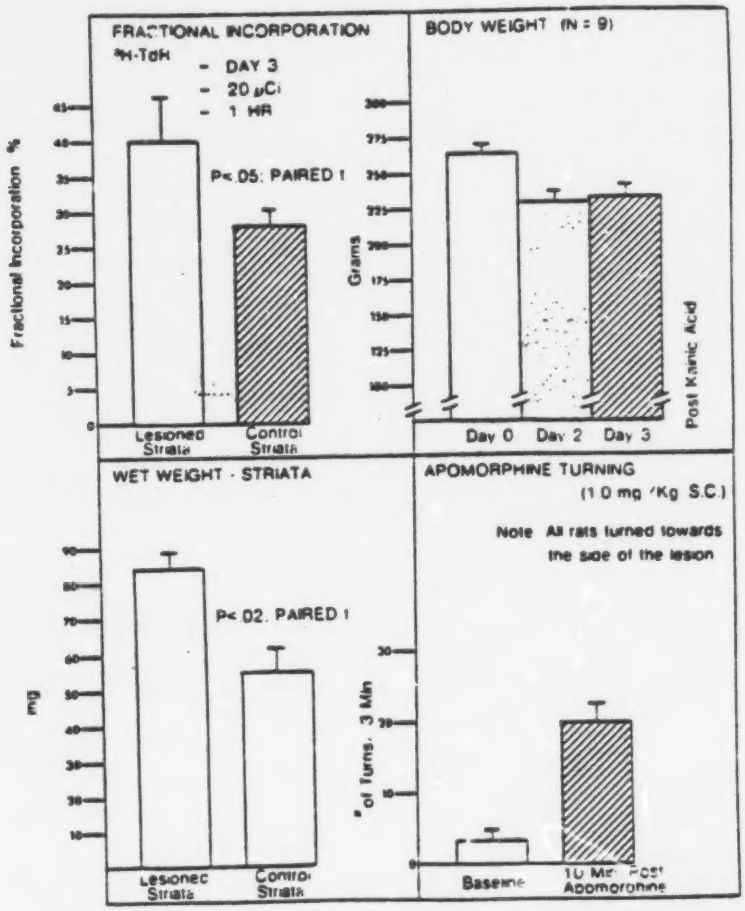

FIGURE 5

STUDIES OF THE POSSIBLE ROLE OF ORNITHINE DECARBOXYLASE IN GLIOSIS ENZYME ACTIVITY AND EFFECTS OF DIFLUOROMETHYLORNITHINE (DFMO) IN LESIONED RAT FRONTAL CORTEX

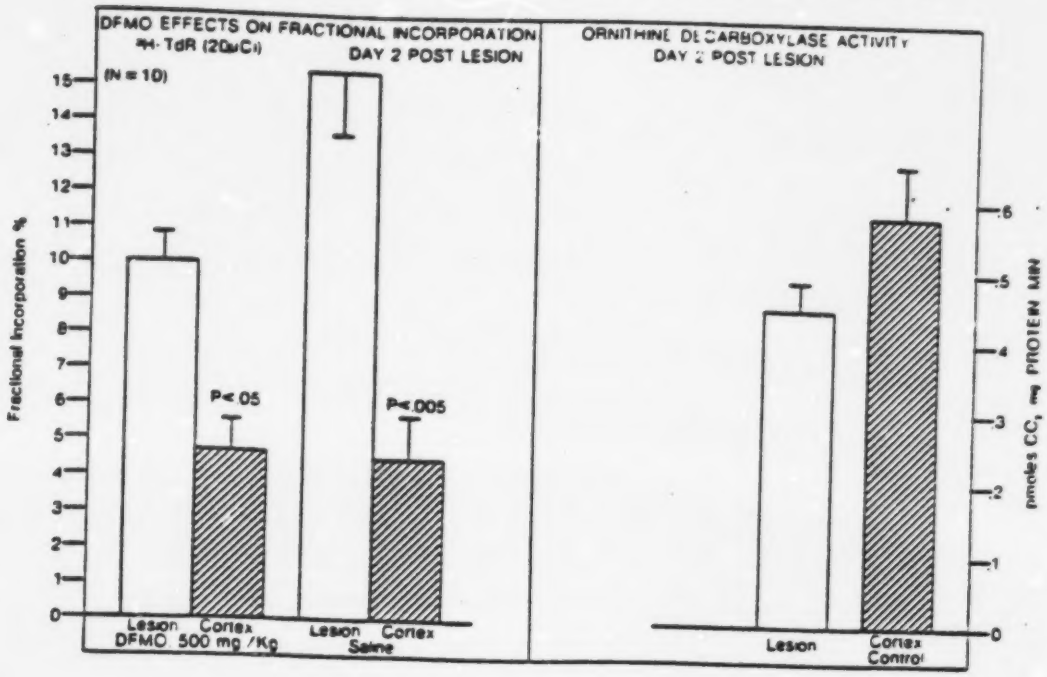

FIGURE 6

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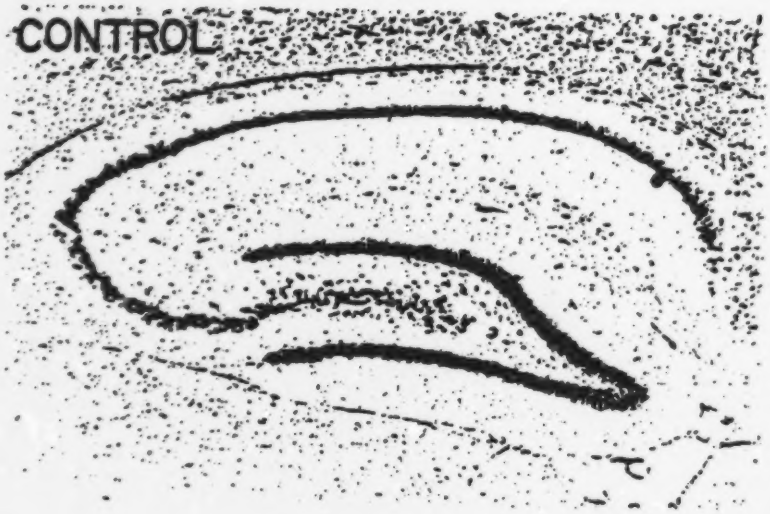


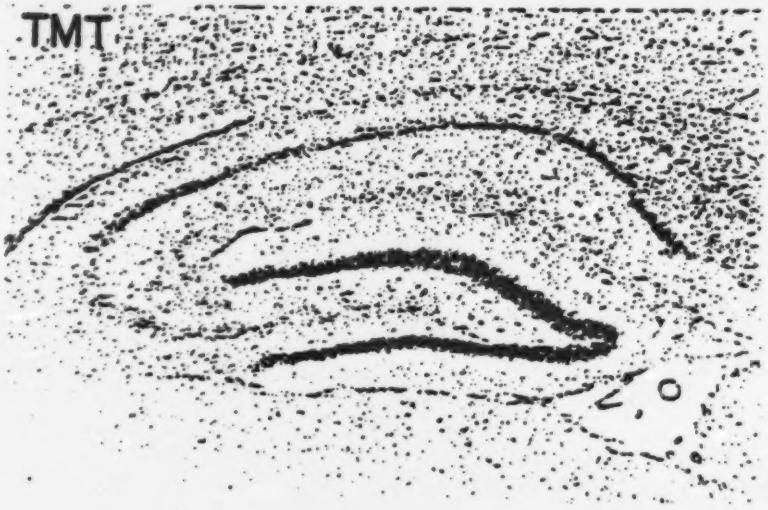


FIGURE 7

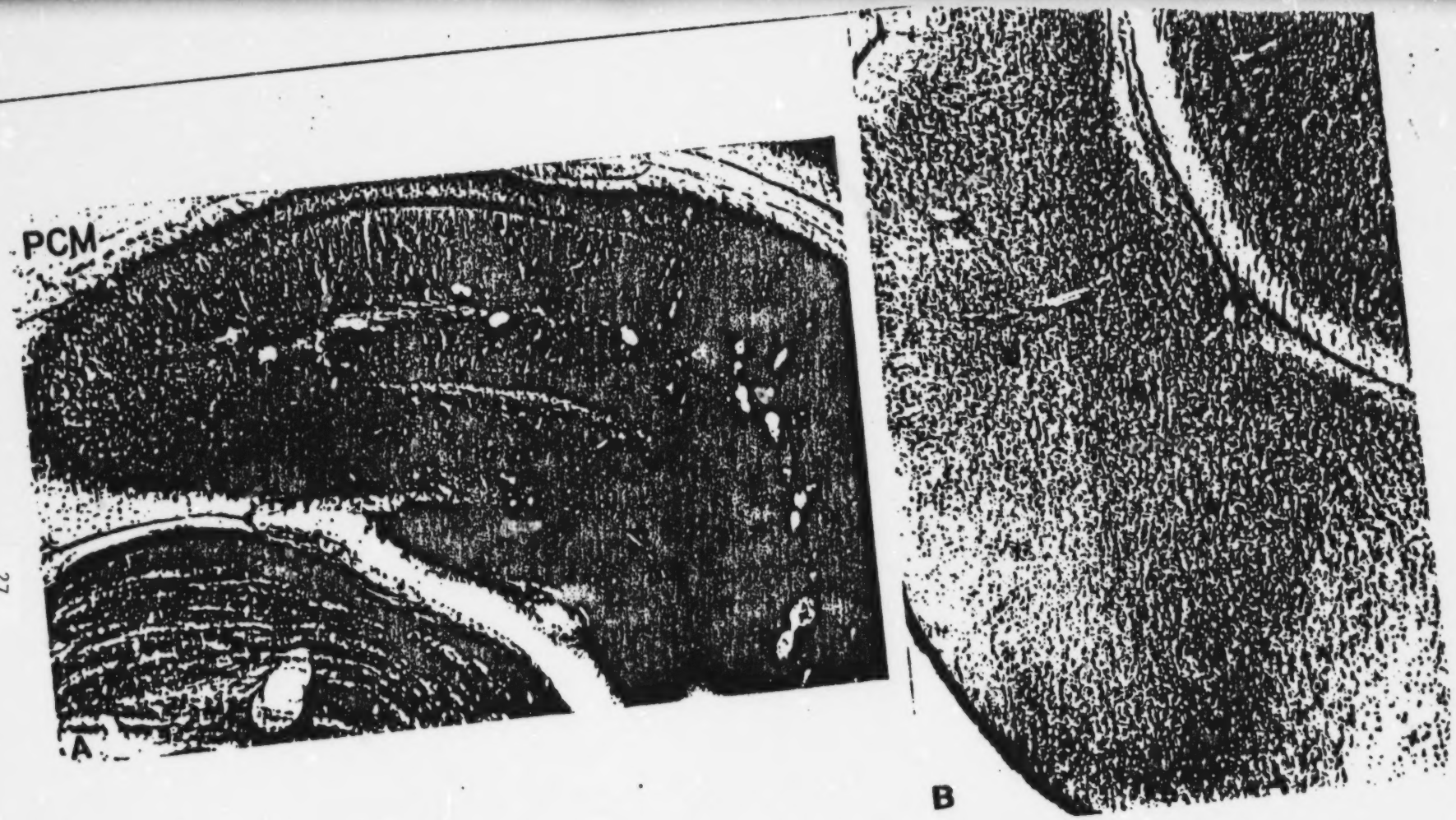
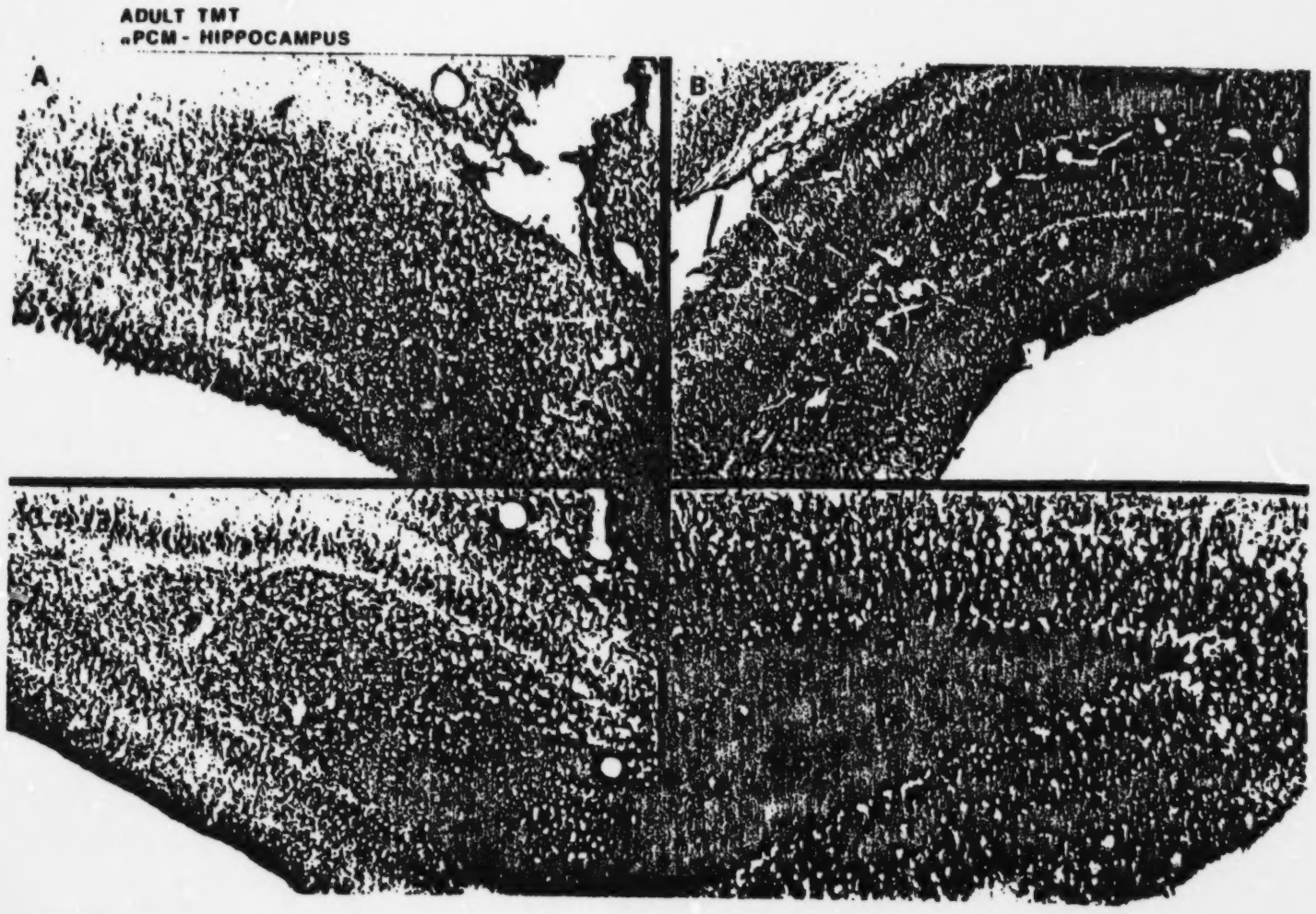


FIGURE 8


ADULT TMT
«PCM - HIPPOCAMPUS
A
B

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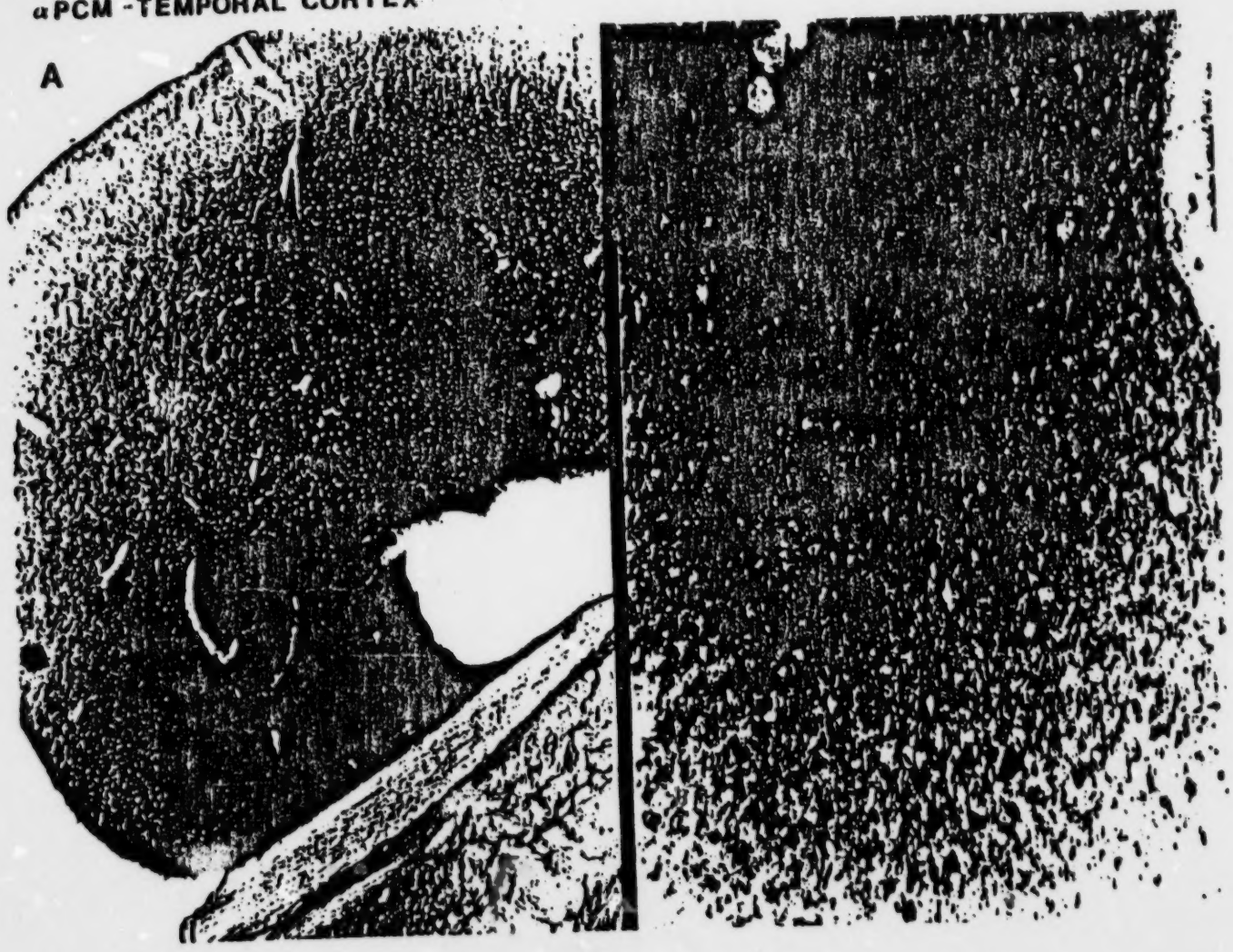

FIGURE 10

SUBSTANTIA NIGRA
α PCM

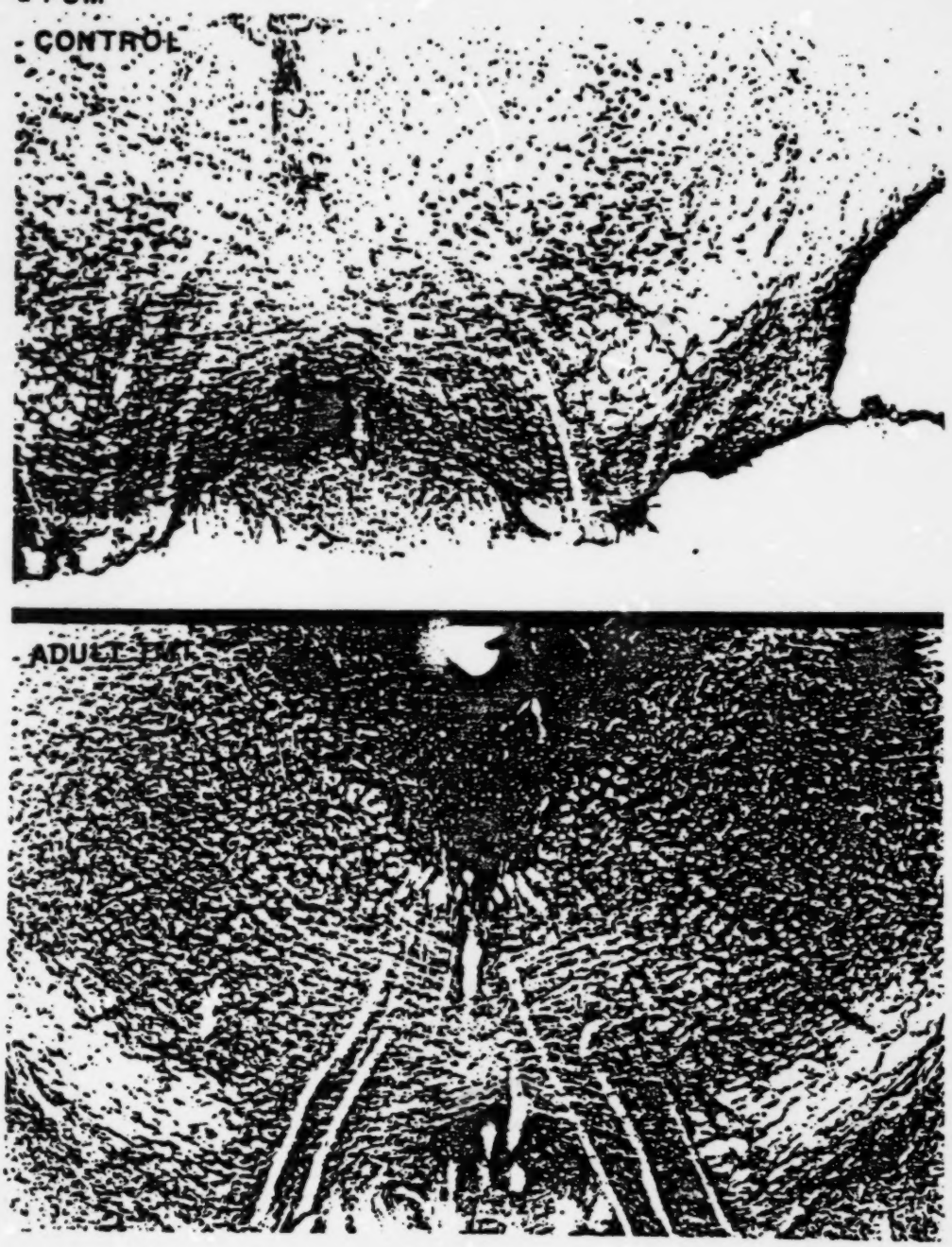

FIGURE 11